8-1-1966

TB23: Studies Concerning the Retention of Organic Materials by Clays

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letin T 23
nical Series

August
1966

ACKNOWLEDGMENT

Acknowledgment is due Jerald Dube of the University of Maine Computer Center for conducting statistical analyses, and to Mrs. Helga Walsh for her assistance in the laboratory.

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David C. Frost and Harold W. Gausman[2]

INTRODUCTION

The influence of organic materials on clays has an important effect on chemical and physical properties of soils. Because of the complex nature of organic matter in the soil, studies of its effect on various soil properties cover a large research area.

The mode of retention of organic materials by clays has an important effect on adsorption and fixation of plant nutrients. Considerable research has been conducted relative to surface adsorption of organic materials by clays, but investigations dealing with interlattice adsorption of organic materials by clay minerals have not been as numerous.

The purpose for conducting research reported herein was to investigate interlattice adsorption of organic materials by montmorillonite clay and clays obtained from Merrimac sandy loam and Suffield silt loam soils.

REVIEW OF LITERATURE

The review of literature will be concerned with interlattice retention and fixation of organic compounds and some inorganic ions by clays.

Retention of Organic Matter by Clays. A number of organic compounds are adsorbed by clay minerals. Meyers (26)[3] reported that there was a physiochemical relationship in the union between organic and inorganic soil colloids. He suggested that attractive forces were due to polar adsorption and that tendencies to combine seemed to be most pronounced under acid soil conditions.

Ensminger and Gieseking (13, 14) found that albumin and gelatin were adsorbed by montmorillonite clays within the expansible portion of the crystal lattice structure of the clays. They also found that these proteins were more completely adsorbed in suspensions with a high hydrogen-ion concentration and that retention of these com-

[1] Part of a thesis submitted by the senior author for an M.S. degree, June, 1965.

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[3] Numbers in parentheses refer to literature citations, page 28.

pounds appeared to be caused by an attraction between basic groups of proteins and negative charges of clays.

Research at the Maine Agricultural Experiment Station (20) indicated that organic materials enter the lattice structure of clay minerals. After removal of all surface-adsorbed organic materials, radioactivity was found in clays previously treated with homogenized bean plants containing S-35 and P-32. Additional studies (21) indicated that pretreatment of clay slurries with plant material reduced the amount of Rb-86 recovered from clay interlayers.

Stevenson and Kidder (35) recently reported that a significant amount of organic matter extracted from soils with hydrofluoric acid occurred within the lattice structure of clay minerals. They found that these compounds were composed mainly of amino acids and amino sugars. Research conducted by Axley (3) demonstrated that organic compounds from several sources, applied to a Meyersville silt loam, entered silt and clay interlayers.

Chin and Wybe (10) found that differences between soil types with respect to urea adsorption were related to their organic matter content.

The organic compounds in soils which occur within clay mineral interlayers were thought to be composed of proteinaceous materials and carbohydrates by Dharival and Stevenson (12). They found that organic materials within clay minerals were not extracted when clays were treated with hot, one normal, KOH for six hours.

Frink (18) found that vinyl pyridine and acrylamide were adsorbed between clay layers and formed polymers. He also found that clay samples contained up to 10 per cent of the polymer. X-ray diffraction measurements of extent of swelling gave an indication that clay sheets were fixed at 14.8 A apart. This left a space of 5.3 A° for the organic polymer since the clay layer itself was 9.5 A° thick.

Walsh and Murdock (38) concluded that ammonium fixation was inhibited by the presence of organic matter in spaces between clay layers which prevented lattice collapse.

Allison et al. (1, 2) reported that types of clay minerals involved in fixation are illite, montmorillonite and vermiculite. Each of these has a 2:1 expandable layer structure which would allow materials to enter and become fixed. Research by Pinck and Allison (30) showed that various quantities of an urease enzyme were completely adsorbed by hydrogen-montmorillonite at all levels, while hydrogen-kaolinite did not adsorb and retain all of the urease applied at any of the levels used.

The composition of soil organic materials has been studied by many

investigators (4, 15, 25, 29, 31, 33, 35) Sorensen (33), for example, conducted research on the decomposition of C-14-labeled barley straw in soil. He found that incorporated C-14 was distributed nearly evenly among fulvic acid, humic acid, and humin.

Retention of Organic Ions by Clays. Frissel and Bolt (19) reported that adsorption of organic ions from herbicides by clays was dependent upon pH and electrolytic concentration of the system. They found that adsorption of acidic herbicides by illite and montmorillonite clays was very high at low pH levels, and at pH 6 to 7 there was no adsorption. The adsorption by kaolinite clay was approximately 20% less at low pH levels, and at pH 5 to 6% there was no adsorption. The adsorption of slightly basic herbicides by all three clays was high at all pH levels studied. At high salt concentrations in the soil solution, adsorption was high due to a probable "salting out" effect.

Ensminger and Gieseking (14) reported that adsorption of proteins by montmorillonite was partly due to the cationic groups in the protein molecule. Additions of an acid increased ionization of amino groups and decreased ionization of carboxyl groups which caused the protein to act as a cation. The opposite effect was noted with addition of a base. The greatest adsorption of proteins by clays occurred when the protein was treated with an acid which enhanced the bonding of basic groups of proteins with negatively charged sites on clay particles.

According to Van Olphen (37), the adsorption of many dipolar organic compounds occurred on layer surfaces and edges of clays. These compounds have an adsorption energy comparable with water and are interchangeable with water on clay adsorption sites. Organic anions are adsorbed on positively charged edges of clay particles (octahedral adsorption), while organic cations are adsorbed on negatively charged face surfaces (tetrahedral adsorption). The extent of adsorption of organic cations is usually greater than the cation exchange capacity of the clays involved, due to van der Waals forces linking hydrocarbon chains of excess organic molecules with adsorbed cations.

Frink (18) treated clays with various gaseous hydrocarbons, and several of them polymerized spontaneously forming a rubber-like coating on the clays. The polymer formation occurred only at the edges of clay sheets.

Axley (3) added ammonium-organic compounds to three different soils and found that ions from these compounds affected the interplanar spacings of the clay and silt minerals.

Retention of Inorganic Ions by Clays. Gieseking (23) reported that large, complex ammonium ions, when added to clay minerals, replaced calcium and hydrogen ions within the expanding lattices of clay minerals.

Using x-ray diffraction patterns, he found that interplanar spacings and intensity of diffracted radiation from these spacings increased with an increase in the amount of complex ion added. This indicated that these ions were adsorbed within the lattices and that the lattice spacings were greater than those of the same clays saturated with smaller cations.

The retention of ammonium ions within the lattice structure of clay minerals has been studied by many investigators (1, 2, 7, 12, 21, 38). The ammonium ion becomes trapped between layers of the 2:1 type clay minerals with an expansible lattice in this type of fixation.

Radioactive Rb-86 has been used to study the retention of ammonium and potassium ions by clay minerals, because of its similar valences and ionic radii (16, 17, 21, 28). There have been conflicting reports as to the effectiveness of Rb-86 as a substitute for the study of potassium and ammonium fixations by clays. Oien et al. (28) found that Rb-86 as RbCl was fixed more strongly in soils than potassium as KCl. They reported that twice as much potassium could be leached from a soil as rubidium. Fried et al. (17) suggested that Rb could be used as a substitute for potassium in studies of soil-plant systems where the nutrient medium is a solution or pure resin form, but it did not appear suitable in a soil system. Franklin and Snyder (16) reported that Rb-86 was bonded more strongly by clays than was potassium, but a linear relationship existed between their respective distributions in a clay system.

Cesium-137 has been used to study the structural relationship of clay minerals (32, 36). Shultz et al. (32) reported that Cs-137 may be irreversibly adsorbed in a manner similar to that of potassium by clay minerals. They found that cesium may be fixed in soils by precipitation on surfaces of micaceous minerals. The cesium incorporated within the crystal structure of clay minerals may be released to some extent by replacement with potassium or ammonium ions.

Tamura (36) indicated that measurements of cesium sorption by 2:1 layer clay minerals could be used as an indicator for detecting the presence of montmorillonite clays. He studied the sorption of Cs-137 by montmorillonite clay which had been previously heated to temperatures up to 900° C. for one hour, and found that maximum retention occurred in clay which had been heated at 600° C. Using four types of clay minerals, it was found that montmorillonite clay sorbed more Cs-137 than the other types of clays. It was postulated that heating caused clay layers to collapse, and upon addition of a solution of 1.5 M $NaNO_3$ containing Cs-137, the layers rehydrate. The sorption of cesium was greater by those clays which did not rehydrate ... it

was shown that cesium sorption was directly related to 10 A° spacings of the clay layers in a collapsed state.

MATERIALS AND METHODS

Investigations were conducted during 1963-65 to determine if organic materials enter the lattice structure of clay minerals. Carbon-14 and S-35 radioisotopes were used to evaluate the retention of organic materials by clays. Clays obtained from Merrimac sandy loam and Suffield silt loam soils and Bentonite clay (montmorillonite) were used in the investigations.

Radioisotopes which were incorporated in organic matter and those which were not will be referred to in this paper as organically combined S-35 or C-14 and inorganic S-35 or C-14, respectively. The clays used in this study will be referred to as Merrimac, Suffield, or montmorillonite clay.

Retention of Rb-86. Clays obtained from Merrimac sandy loam and Suffield silt loam soils by sedimentation using Stoke's law (5) were used to study the retention of Rb-86. The procedure used was a modification of Tamura's (36).

One gram samples of each clay were placed in porcelain crucibles and heated for one hour in a muffle furnace at temperatures of 200, 400, 600, and 800° C. followed by drying in a dessicator. An unheated 1-gram sample of each clay was also dried in a dessicator at room temperature (20° C.). Next, 10 ml. of Rb-86 solution were added to each crucible. These samples were stirred at intervals over a one hour period, and then they were filtered into 50 ml. volumetric flasks and washed with 30 ml. of distilled water. The volumetric flasks were then brought up to a volume of 50 ml. with distilled water. A standard sample was prepared by filtering 10 ml. of the Rb-86 solution into a 50 ml. volumetric flask, washing with 30 ml. of distilled water and bringing the solution up to volume. Two ml. aliquots were removed from each flask for radioactivity measurements using a model ED-8, well-type, scintillation counter with a model SC-90, utility scaler, manufactured by Tracerlab, Inc., Waltham, Massachusetts. The above procedure was used for each of two trials conducted on February 20 and March 1, 1963. The concentrations of Rb-86 solution added to the clays were 7.64×10^{-4} and 6.64×10^{-4} microcuries per ml., respectively.

Autoradiographic studies were conducted on retention of Rb-86 by montmorillonite clay to investigate possible interlattice retention or fixation of Rb-86. Thirty ml. of Rb-86 solution were added to a 1-gram

sample of clay and the resulting slurry was left at room temperature for 48 hours with frequent stirring. Water-soluble, surface-adsorbed, and interlattice fractions of Rb-86 were removed from the clay using a procedure outlined by Dharival and Stevenson (12) for the determination of fixed ammonium. The water-soluble fraction was removed by filtering the clay—Rb-86 slurry and washing with 30 ml. of distilled water. The surface-adsorbed fraction was removed by adding 30 ml. of 1.0 N KOH to the clay residue remaining after removal of the water-soluble fraction and simmering on a hot plate for 6 hours. The clay slurry was centrifuged and washed three times with 30 ml. of 0.5 N KCl. The remaining residue was then treated with 30 ml. of a solution containing equal parts of 5.0 N HF, 0.6 N H_2SO_4, and 0.75 N HCl to remove interlattice of fixed Rb-86. After 16 hours the clay-acid mixture was centrifuged and washed three times with 30 ml. of distilled water.

One drop of each clay residue remaining after removal of surface-adsorbed and interlattice fractions of Rb-86 was placed on a separate glass slide and dried at room temperature. The slides were placed under an Anscochrome, non-screen, x-ray film in a medical cassett. After three days exposure, the film was developed in Kodak rapid x-ray developer for 6 to 7 minutes, rinsed in water containing a few drops of acetic acid, and fixed in Kodak acid fixer for 15 minutes. After washing with water for 20 minutes, the x-ray film was air dried.

Retention of S-35 and C-14. The retention of organically combined S-35 and C-14 and inorganic S-35 and C-14 by unsterilized and sterilized clays was investigated by measuring the radioactivity remaining in clay residues after removal of various fractions. The procedures used for this study are given below:

Additions of Radioisotopes to Bean Plants

Kentucky Wonder bean plants grown in the greenhouse in one-gallon, glazed crocks containing quartz sand were used as a source of organic matter for this study. The plants were fed daily with 500 ml. of Hoagland's nutrient solution (24) by surface irrigation; excess solution was allowed to drain into 500 ml., amber-colored Erlenmeyer flasks. Solutions were brought up to 500 ml. volume each day with distilled water. Nutrient solutions were changed each week. When the plants were 5 weeks old, one millicurie of S-35 in the form of $H_2S^{35}O_4$ was added to each nutrient solution of three plants. Prior to adding S-35, the plants were not watered for 48 hours. The nutrient solution containing S-35 was poured back into the crocks several times throughout the day. After 24 hours, each plant was harvested and

dipped into a 1% Chlorox solution for two minutes, rinsed with sterile, deionized water, and homogenized in a sterilized Waring blender for five minutes. The homogenate was then stored in sterile cartons in a refrigerator at a temperature of —20° C.

Three plants were treated with C-14 using a method described by Comar (11). A crock containing the bean plant was placed inside a bell-jar which was sealed. Prior to placing the bell-jar over the plant, four ml. of 60% perchloric acid was placed in a small glass vial that had a hole in the bottom which was plugged with filter paper. The vial was placed upright in a small beaker containing 0.33 millicuries of C-14 as $Na_2C^{14}O_3$, which was also placed inside the bell-jar. The acid eroded the filter paper plug, drained out of the vial, and reacted with the $Na_2C^{14}O_3$, thus liberating $C^{14}O_2$ which was available for assimilation by the plant. The plant was left in the bell-jar for 24 hours. Procedures for harvesting and storing plants treated with C-14 were the same as for S-35.

Autoradiographs were prepared to show that radioactivity was present in plants treated with S-35 and C-14.

Clay Treatments. After thawing the frozen homogenized plant material containing S-35 or C-14 at room temperature, approximately 50 ml. of homogenate were added to 2-gram samples of each clay. Water was added to each sample to form a slurry or thin paste. The samples were then left at room temperature for 40 hours with frequent stirrings.

After incubating for 40 hours, the six samples were subjected to treatments to remove water-soluble, surface-adsorbed, and interlattice fractions of added materials from clays using modifications of methods given by Dharival and Stevenson (12). Three particle size separations were made of the clay residue remaining after removal of the last fraction. The procedures used to separate the fractions and particle sizes are outlined below.

1. The clay surrey from each sample was centrifuged and washed three times with 20 ml. of distilled water to remove the water-soluble fraction as a supernatant liquid.
2. The adsorbed fraction was removed as a supernatant liquid, by treating each clay residue with 100 ml. of 1.0 N KOH, simmering on a hot plate for 6 hours, centrifuging, and then washing and centrifuging the residue three times with 30 ml. of 0.5 N KCl.
3. Thirty ml, of HF solution containing equal parts of 5.0 N HF, 0.75 N HCl, and 0.6 N H_2SO_4 was added to each of the remaining clay residues to remove the interlattice fractions. After standing 16 hours with frequent stirring, the HF-soluble fraction

was removed, as a supernatant liquid, by centrifuging and then washing and centrifuging the residue three times with 30 ml. of distilled water.

4. Three particle size separations were prepared from each clay residue remaining after removal of the HF fraction by suspending the residues in water and centrifuging at a speed of 3,000 rpm (1,000 × gravity) for five minutes. The supernatant fractions from this and a subsequent centrifugation were centrifuged at 9,000 rpm (9,000 × gravity) and 15,000 rpm (25,000 × gravity).

Duplicate samples of approximately one ml. of the clay residue, remaining as a thick slurry, after removal of each of the above fractions and particle size separations, were placed in numbered, preweighed metal planchets and dried under an infra-red heat lamp. The samples were stored for counting. This procedure was used for each of three runs for both S-35 and C-14.

Microscopic examinations of montmorillonite clay residues separated by centrifugation were conducted to observe differences in particle size or conglomerations of the clay, and to associate them with recovered radioactivity. Observations were made with a light microscope at 450 X. Quadrafos was used to disperse the clay. Photomicrographs were made using a model 95 Polaroid camera and a model ACA Unitron photomicrography apparatus.

The procedures outlined above were also used for experiments using S-35 and C-14 to investigate their retention by clays which had been sterilized with CCl4. The calys were spread in thin layers over filter papers supported by a wire mesh screen inside a metal box. Carbon tetrachloride was placed in a large beaker under the wire screen with paper toweling placed in the beaker to increase the evaporating surface. The clays were left in contact with the CCl_4 vapors for 48 hours and then stored in sterilized metal containers until ready for use.

Radioactivity Measurements. Measurements of radioactivity in duplicate samples of clay residues containing either S-35 or C-14 were made using a flow-counter manufactured by Radiation Equipment and Accessories Corporation with a model E-120 scaler which was manufactured by Oxford Engineering Corporation.

Procedures used for determining counter operating voltage and corrections for radioactive decay, self-adsorption, background activity, machine efficiency, and sample weights were those given by Chase and Rabinowitz (9). An outline of the procedures used to correct sample counts is given below:

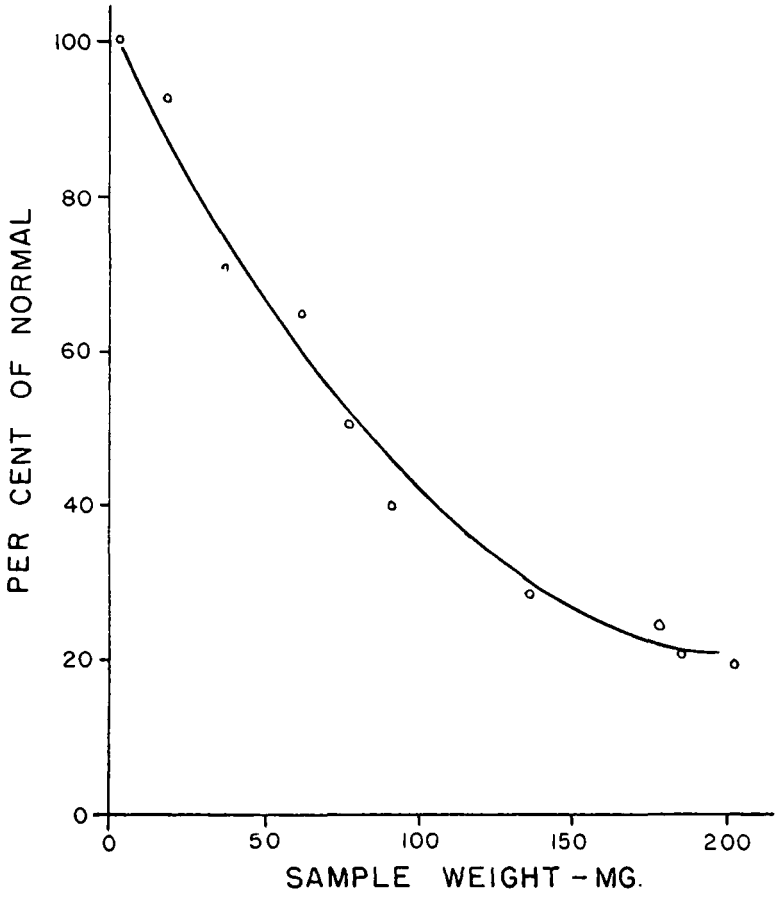

FIGURE 1—Calibration curve for self-absorption of radio activity by samples of montmorillonite clay after removal of the water-soluble fraction of organically combined S-35.

1. The net weight of each sample was recorded in mg.
2. Radioactivity was determined in counts per minute, cpm, by averaging ten minute counts of each sample.
3. Background activity was determined each day, and that count was subtracted from the daily sample counts.
4. Corrections for radioactive decay were made on all sample counts except those containing C-14, because of its long half-life.
5. Sample counts were then calculated to cpm per 100 mg. of sample.

6. Corrections for absorption of radiation by samples were made on each sample count using calibration curves for self-absorption. These curves were constructed for each combination of clay, fraction, and form of S-35 and C-14 used. Such corrections were necessary to standardize each sample count to a comparable sample weight or thickness. A typical calibration curve for self-absorption, which was constructed from data presented in appendix table 1[4], is shown in figure 1. Data used to construct the other curves are not included.

7. Sample counts were corrected for machine efficiency by comparing the observed activities of a known amount of S-35 and C-14 with calculated disintegrations per minute.

Electrophoresis Studies. The presence of sulfur-containing amino acids in the interlattice fractions of montmorillonite clay was tested by procedures given by the Gelman Instrument Company, Ann Arbor, Michigan, manufacturer of the apparatus used (22). Samples of supernatant liquid after HF solution treatment to montmorillonite clay were applied to sepraphore cellular acetate strips which had been soaked for 8 hours in a 6% formic acid solution. Separations were conducted at 250 volts for 30 minutes using a formic acid buffer solution and autoradiographs were prepared.

Statistical Analyses. Statistical analyses were conducted using methods given by Steel and Torrie (34). Least significant differences were computed for treatment means of Rb-86 retention data at the 5½% levels of probability. Duncan's New Multiple Range Test was used at the 5% probability level to compare treatment means and their interactions for the S-35 and C-14 experiments.

All statistical analyses of logarithmic-transformed, S-35 and C-14 data were conducted at the University of Maine Computer Center on an IBM 1620 Computer.

RESULTS AND DISCUSSION

The results and discussion are limited to statistically significant results. The terms significant and highly significant are used to indicate statistical significance at 5% and 1% probability levels, respectively.

Retention of Rb-86 by Differentially Heated Clays. Studies were conducted on lattice collapse of clay minerals involving the retention of Rb-86 by clays obtained from Merrimac sandy loam and Suffield silt loam soils and differentially heated. It has been reported that vermicu-

[4] Appendix tables are presented on page 30.

lite is the dominant clay mineral in these two soils, but clay from the Merrimac soil contained material that prevented lattice collapse to 10 A° after K^+ saturation (27). Since Rb-86 and K^+ have similar ionic radii and their valences are the same, Rb-86 has been used as a substitute for K^+ in adsorption and fixation studies (16, 17, 21, 28).

Data obtained from two trials on retention of Rb-86 by heated Merrimac and Suffield clays are presented in tables 2 and 3 of the appendix. A summary table of the analysis of variance for these data is presented in appendix table 4. Data are presented in table I for the highly significant interaction of clays with temperature changes.

The greatest average retention of Rb-86, 98.4% occurred at a temperature of 400° C. and the lowest, 36.9%, at 800° C. Compared with the control, there was a highly significant difference in mean percentage of retention at all temperatures except 200° C., which was significantly different at the 5% probability level. There was no significant variation between clays in Rb-86 retention. There was, however, a highly significant interaction between clays and temperature in that the difference in Rb-86 retention between clays was −21.1 and +23.8% at 600 and 800° C., respectively. The reason for the greater retention of Rb-86 by Suffield clay at 600° C. and by Merrimac clay at 800° C. is unknown. Nevertheless, these results indicate that vermiculitic clays in the Merrimac and Suffield soils reacted differently or that differences existed in the composition of Merrimac and Suffield clays.

These results are in accord with those obtained by Tamura (36) on the retention of Cs-137 by heated clays. He found that retention

Table I—Retention of Rb-86 by clays which were obtained from Merrimac and Suffield soils, and differentially heated, average of two trials, March, 1963.

Temperature	Clays		Mean	Difference
	Merrimac	Suffield		
C.	%	%	%	%
20 (control)	86.6	91.6	89.2	− 5.0
200	95.2	94.0	94.6	+ 1.2
400	96.0	99.2	98.4	− 3.2
600	66.8	87.9	78.3	−21.1**
800	49.2	25.4	36.9	+23.8**
L.S.D. 5%			4.92	
1%			7.07	

** Denotes significance between clays at a given temperature for the 1% probability level.

of Cs-137 reached a maximum at 600° C., and that montmorillonite clays retained more Cs-137 than did three other types of clay minerals studied. He suggested that cesium sorption tests could be useful as an indication of the presence of montmorillonite clay minerals. He also related cesium sorption directly to 10 A° spacings of the clay layers in a collapsed state.

Autoradiographic Studies with Rb-86. The retention of Rb-86 by montmorillonite clay, after removal of water-soluble, surface-adsorbed, and interlattice fractions, was studied by using autoradiographs to detect the presence of Rb-86 in clay residues after removal of the three fractions. As shown in figure 2, radioactivity was present in clay residues after removal of surface-adsorbed and interlattice fractions of Rb-86. This is an indication that Rb-86 was fixed within the lattice structure of the montmorillonite clay.

Retention of Inorganic S-35 and Organically Combined S-35. The retention of organic materials by samples of montmorillonite, Merrimac, and Suffield clays was studied using S-35-tagged bean plants as a source of organic matter. Inorganic S-35 was used as a control treatment. A photograph of an x-ray film showing radioactivity in bean leaves from a plant used in this study is presented in figure 3. The

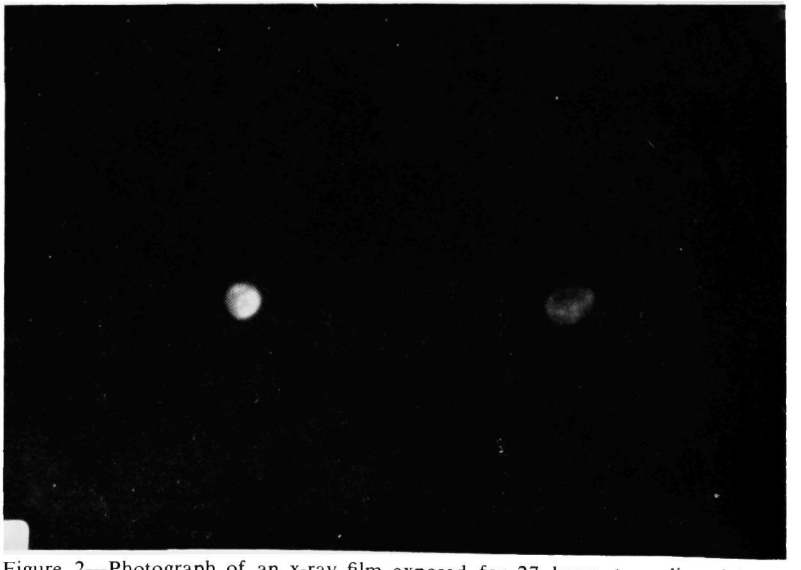

Figure 2—Photograph of an x-ray film exposed for 27 hours to radioactivity in clay residues after removal of surface-adsorbed Rb-86 on left, and interlattice fractions of Rb-86 on right, March, 1963.

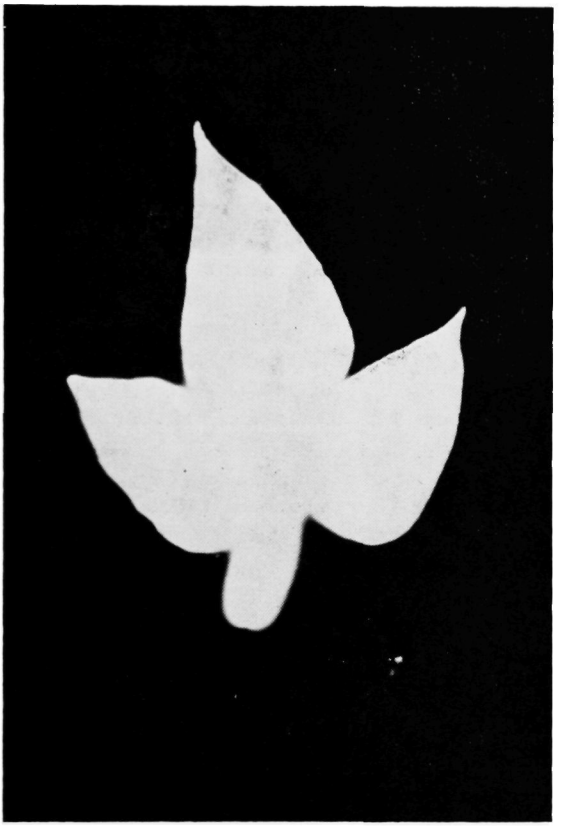

FIGURE 3—Photograph of an x-ray film exposed for 24 hours to radioactivity in bean leaves from a S-35-tagged plant, December, 1963. White area indicates radioactivity.

white area indicates an abundance of radioactivity after an exposure to the x-ray film of only 24 hours.

Data obtained for the retention of organically combined S-35 and inorganic S-35 by samples of the three clays are presented in appendix tables 5 and 6. These data are an average of three runs using duplicate samples. The analysis of variance for these data is given in appendix table 7.

There was no significant variation between clays in retention or organically combined S-35 and inorganic S-35. There was, however, a highly significant variation in retention of both forms of S-35 due to fractionation into various clay residues. There was a highly signifi-

cant variation between treatments or retention of organically combined S-35 and inorganic S-35 and between the interaction of these two treatments with clay residues.

Data for the interaction of clay residues with organically combined S-35 and inorganic S-35 are presented in table II as an average of three runs for three clays using duplicate samples. Differences in retention between H_2O and KOH residues are a measure of surface adsorption, and differences between KOH and HF residues are an evaluation of interlattice retention. Differences between HF_1, HF_2, and HF_3 represent the association of retention of S-35 with decreasing particle size of clay residues.

Table II—Retention of S-35 by six clay residues, average of three runs with three clays using duplicate samples, 1964.

Residues	Treatments: Organically combined S-35	Treatments: Inorganic S-35	Mean[1]	Difference
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	164,859	269,863	217,361a	−105,004
KOH	81,404	175,476	128,440a	− 94,072*
HF	39,468	3,934	21,701b	+ 35,534**
HF_1	13,723	2,554	8,138c	+ 11,169
HF_2	10,599	2,626	6,612cd	+ 7,973**
HF_3	3,609	2,047	2,828d	+ 1,562**

[1] Means followed by common subscripts are not significantly different at the 5% probability level.

*,**Denotes significant difference between treatment means at the 5 and 1% probability levels, respectively.

Duncan's New Multiple Range Test was used to test differences between main effects of clay residues at the 5% probability level. There was a decrease in retention of S-35 as fractions were removed from the clays and as particle size decreased. Significant differences existed between all means except H_2O and KOH, HF_1 and HF_2, and HF_2 and HF_3. A difference of 106,739 cpm/100 mg. occurred between KOH and HF which indicates that interlattice retention occurred. Photomicrographs of montmorillonite clay residues from HF_1, HF_2, and HF_3 particle size separations are shown in figure 4. As indicated, there was a decrease in particle size with increased speed of centrifugation. Relative to the HF_1 and HF_3 clay residues, there was a significant decrease in retention of S-35 with a decrease in particle size.

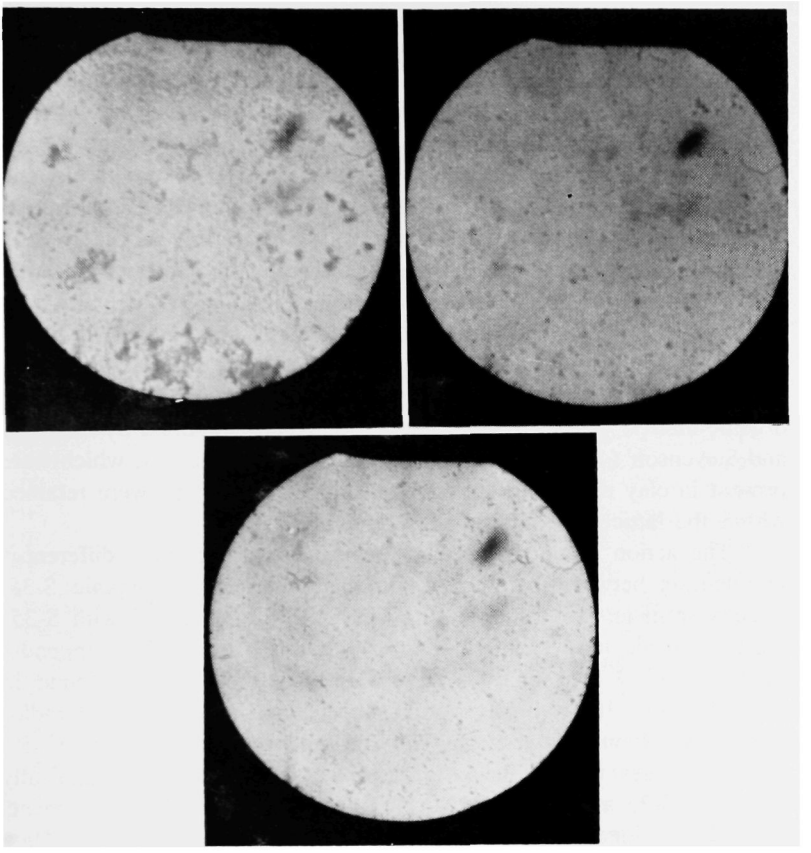

Figure 4—Photomicrographs of montmorillonite clay residues after separation by centrifugation; upper left — 1,000 x G, upper right — 9,000 x G, lower — 25,000 x G, November, 1964.

The presence of radioactivity in the smaller sized clay particles, however, is an indication that S-35 was contained within the lattice structure of the clay.

As shown in table II, significant differences between retention of organically combined S-35 and inorganic S-35 varied with clay residues. An analysis of variance for each residue showed that differences in S-35 treatments were significant for all HF fractions with the retention of organically combined S-35 being higher than inorganic S-35. The retention of inorganic S-35 was significantly greater than organically combined S-35 for KOH clay residues, amounting

to 94,072 cpm/100 mg. The differences between S-35 treatments were not significant for H_2O.

These results show that retention of organically combined S-35 by clay residues after removal of interlattice fractions was greater than inorganic S-35, but the retention of inorganic S-35 was greater than organically combined S-35 after removal of water-soluble and surface-adsorbed fractions. This would indicate that KOH treatments did not remove all adsorbed inorganic S-35 or possibly inorganic S-35 was retained within clay lattice structures. Since the amounts of radioactivity present in clay residues after removal of interlattice fractions were greater where organically combined S-35 was added, it would seem that KOH treatments did not remove all the adsorbed inorganic S-35 and that there was a greater interlattice retention of S-35-tagged organic matter. This is in agreement with results obtained by Dharival and Stevenson (12). They assumed that organic materials, which were present in clay residues after removal of KOH fractions, were retained within the lattice structure of clay minerals.

The action of microorganisms may account for some differences in retention between organically combined S-35 and inorganic S-35. Results from an experiment using sterilized clays treated with S-35-tagged organic matter and inorganic S-35 are presented in appendix tables 8 and 9. The analysis of variance for these data is found in appendix table 10. A highly significant variation in retention of radioactivity was found between clay residues and interaction of these with the three clays used. The variance due to treatments of organically combined S-35 and inorganic S-35, and interaction of these treatments with clay residues was significant at the 5% probability level. Data for this interaction is presented in table III as an average of three clays and duplicate samples. There was no significant difference in average retention by clay residues between H_2O and KOH, or between HF_2 and HF_3. Differences in retention between all other means were significant.

There was an interaction of clay residues with S-35 treatments in that retention of organically combined S-35 was significantly greater than inorganic S-35 for clay residues after removal of HF and HF_1 fractions at the 1% probability level. These differences amounted to 12,864 and 14,253 cpm/100 mg., respectively.

These results follow the same trend as those presented in table II for the retention of S-35 by unsterilized clays, although a comparison of the two tables indicates that retention of inorganic S-35 was not as great by sterilized clay residues after removal of interlattice fractions

and particle size separations. For example, retention of inorganic S-35 by sterilized clay residues was 1,324 cpm/100 mg. less than unsterilized clay residues after removal of HF fractions. Conversely, more S-35 was retained by residues of sterilized clays as compared with unsterilized clays after removal of water-soluble and surface-adsorbed fractions.

Table III—Retention of S-35 by six residues of sterilized clays, average of three clays using duplicate samples, 1964.

Residues	Treatments		Mean[1]	Difference
	Organically combined S-35	Inorganic S-35		
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	117,580	272,393	194,987 a	−154,813
KOH	86,790	136,399	111,594 a	− 49,609
HF	15,474	2,610	9,042 b	+ 12,864**
HF_1	12,691	1,562	7,121 c	+ 14,253**
HF_2	8,819	2,329	5,574 d	+ 6,490
HF_3	5,325	1,112	3,218 d	+ 4,213

[1] Means followed by common subscripts are not significantly different at the 5% probability level.

** Denotes significant difference between treatment means at the 1% probability level.

Results indicate that organic matter is retained within clay interlayers; this supports the findings of Dharival and Stevenson (12) and Stevenson and Kidder (35). They reported that organic matter could be extracted from clays with HF solution after removal of surface-adsorbed materials by hot, one-normal KOH. Research by Axley (3) indicated that organic materials from various plant sources entered silt and clay interlayers. He based his conclusion on x-ray diffraction measurements. Several other investigators have also reported the interlattice adsorption and retention of organic materials by clay minerals (13, 14, 18, 20, 21).

The retention of inorganic S-35 by clay residues after removal of surface-adsorbed and interlattice fractions would tend to indicate that S-35 was also retained within clay interlayers. Chao et al. (8) reported that 45% of sulfur adsorbed by soils could be removed by water extraction. The remainder was retained by surface adsorption or fixation. Results from studies reported herein indicate that S-35 was retained by both surface adsorption and interlattice fixation. More important, there was greater retention of S-35 in combination with organic matter than in the inorganic form.

Retention of Inorganic C-14 and Organically Combined C-14. Experiments were conducted using bean plants which had been tagged with C-14 as a source of organic matter to study retention of organic materials by clays. A photograph of an x-ray film showing radioactivity present in bean leaves which had been tagged with C-14 is presented in figure 5. This is evidence that radioactivity was present in the plant tissue.

Results from three runs for retention of organically combined C-14 and inorganic C-14 by duplicate samples of clay residues are presented in appendix tables 11 and 12. The analysis of variance for these results, given in appendix table 13, shows that there was a

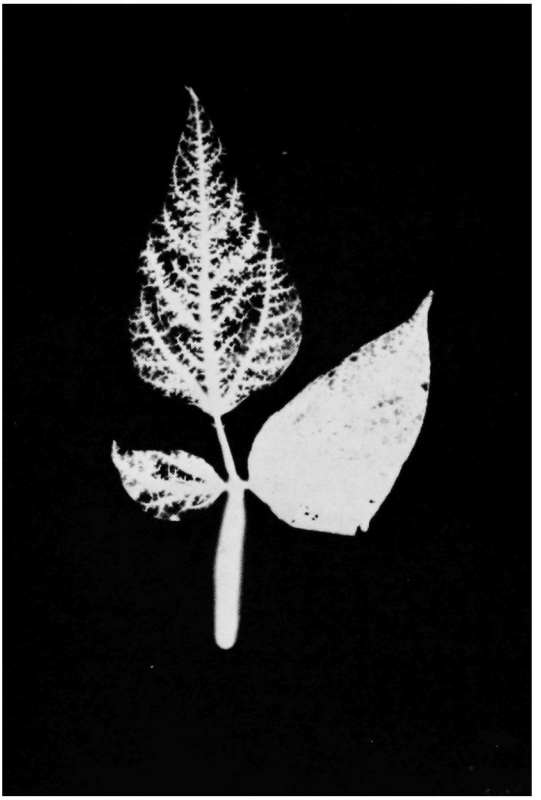

FIGURE 5—Photograph of x-ray film exposed 24 hours to radioactivity in bean leaves from C-14-tagged plant, December, 1963. White areas indicate radioactivity.

significant difference between clay residues. The interaction of residues with C-14 treatments was also highly significant. The data for this interaction is presented in table IV as an average of three clays and duplicate samples.

Duncan's test showed that the differences between H_2O and KOH, HF and HF_1 and HF_1 and HF_2 were not significantly different at the 5% probability level. All other means of C-14 retention by clay residues were significantly different from each other. These comparisons show that there was not a significant difference between amounts of surface-adsorbed and interlattice fractions of C-14, although there was a significant difference of 68,294 cpm/100 mg. between average amounts removed by KOH and HF which represents interlattice adsorption. There was a difference of 2,183 cpm/100 mg. in retention between HF and HF_3 which indicates that larger clay particles retained more C-14 than smaller particle sizes. This is evidence that organic materials are contained within clay interlayers.

Table IV—Retention of C-14 by six clay residues, average of three runs with three clays using duplicate samples, 1964.

Residues	Treatments: Organically combined C-14	Treatments: Inorganic C-14	Mean[1]	Difference
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	7,927	139,345	73,636 a	−131,418**
KOH	4,972	138,514	71,743 a	−133,542**
HF	4,916	1,983	3,449 b	2,933**
HF_1	3,548	1,030	2,289 bc	2,518**
HF_2	2,788	980	1,884 c	+ 1,808**
HF_3	1,676	856	1,266 d	+ 820*

[1] Means followed by common subscripts are not significantly different at the 5% probability level.

** Denotes significant difference between treatment means at the 5% and 1% probability levels, respectively.

Differences in retention by clay residues between organically combined C-14 and inorganic C-14 were highly significant for each residue except HF_3 which was significant at the 5% probabality level. Retention of inorganic C-14 was greater than organically combined C-14 by clay residues after removal of water-soluble and surface-adsorbed fractions, while retention of organically combined C-14 was greater for remaining clay residues. As with S-35 data, this indicates that

more inorganic C-14 was initially retained by surface and interlattice adsorption or that KOH treatment did not remove all surface-adsorbed, inorganic C-14. The retention of organically combined C-14 was 2,933 cpm/100 mg. greater than inorganic C-14 after HF removal and retention was also greater in residues after particle size separations, which indicates that C-14-tagged organic matter was retained within clay interlayers.

Results of data obtained from retention measurements using sterilized clays are presented in appendix tables 14 and 15 as an average of duplicate samples. The analysis of variance for these results is given in appendix table 16. The only significant variance was due to retention by clay residues after removal of various fractions. The interaction of clay residues with C-14 treatment is presented in table V as an average of three clays and duplicate samples.

As shown by Duncan's test, there were no significant differences between HF and HF_1, HF_1 and HF_2, or HF_2 and HF_3. All other mean comparisons were significantly different. A noticeable difference was observed between results of this experiment as compared to those presented in tables II, III, and IV in retention after removal of KOH or surface-adsorbed fractions. In this experiment there was a significant reduction of 55,739 cpm/100 mg. in average retention of C-14 after removal of KOH fractions, as compared with the H_2O fraction, while in others there were no significant reductions. This may be an indication that sterilization had some effect on surface adsorption of C-14-containing materials by clays. It is also possible that microbial activity

Table V—Retention of C-14 by six residues of sterilized clays, average of three clays using duplicate samples, 1965.

Residues	Treatments		Mean[1]	Difference
	Organically combined C-14	Inorganic C-14		
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	12,620	111,000	61,810a	−98,380
KOH	5,598	6,544	6,071b	− 946
HF	1,949	876	1,412c	+ 1,073
HF_1	1,301	513	907cd	+ 788
HF_2	919	342	630de	+ 577
HF_3	483	479	481e	+ 4

[1] Means followed by common subscripts are not significantly different at the 5% probability level.

enhanced retention of C-14 compounds in experiments where unsterilized clays were used.

Results obtained using C-14 are similar to those obtained using S-35 in that retention of inorganic C-14 was greater than organically combined C-14 by clay residues after removal of water-soluble and surface-adsorbed fractions, but was less after removal of interlattice fractions. This is further evidence that organic matter is retained by clay interlattice structures.

Electrophoresis Studies. Electrophoretic separations of interlattice fractions of S-35-tagged organic matter from montmorillonite clay have indicated that S-35-containing amino acids were present. A photograph

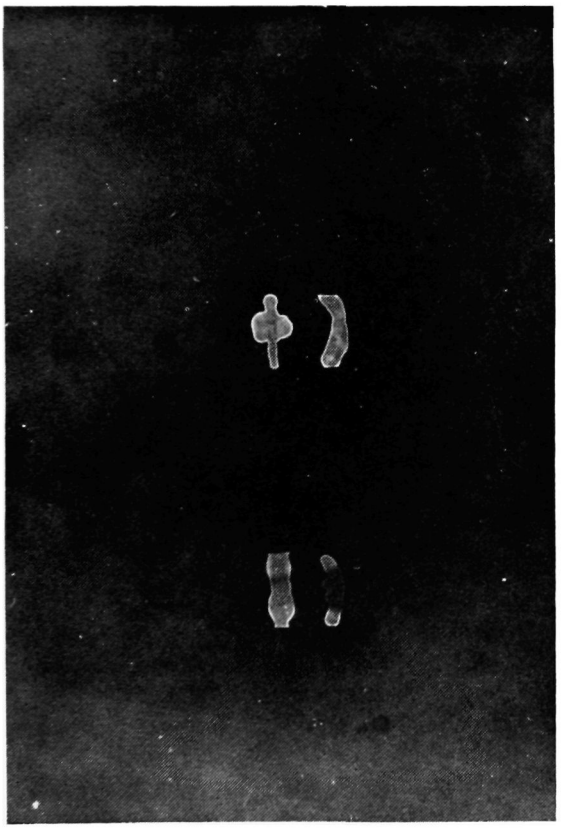

FIGURE 6—Photograph of x-ray film exposed for 33 days to S-35-cotaining amino acids separated, on duplicate electrophoresis strips, from interlattice fractions of tagged organic matter, 1964

of an x-ray film which was prepared to detect S-35-containing amino acids on duplicate electrophoresis strips is presented in figure 6. As shown, radioactivity was present in two of the fractions. No attempt was made to identify the S-35-containing amino acids separated in this study.

The presence of amino acids in interlattice fractions of organic matter removed from the clay substantiates investigations of Stevenson and Kidder (35) who reported that clay interlayer materials consisted chiefly of amino acids and amino sugars.

SUMMARY

Laboratory investigations were conducted on the effect of heating clays, obtained from Merrimac sandy loam and Suffield silt loam soils, on retention of Rb-86. Retention of Rb-86 by montmorillonite clay was also studied using autoradiographs to measure radioactivity remaining in clay residues after each removal of surface-adsorbed and interlattice fractions of Rb-86.

Sulfur-35 and C-14-tagged bean plants, and inorganic S-35 and C-14 were used to evaluate retention of organic materials by three sterilized and unsterilized clays after each removal of three fractions and three clay particle size separations. Montmorillonite clay and clays obtained from Merrimac and Suffield soils were used. Electrophoretic separations of amino acids were conducted on interlattice fractions of S-35-tagged organic matter.

Results obtained from these studies are:

Heating clays from Merrimac and Suffield soils to 200° and 400° C. resulted in greater retention of Rb-86 compared with unheated clays, but heating to 600° and 800° C. resulted in decreased retention of Rb-86. There was a highly significant difference in retention of Rb-86 between the two clays at 600° and 800° C. which indicates that there were differences in the types of clay minerals contained in the Merrimac and Suffield soils.

Radioactivity was detected by autoradiographs in montmorillonite clay residue after removal of water-soluble, surface-adsorbed, and interlattice fractions of Rb-86, which indicates that Rb-86 was fixed within the lattice structure of the clay.

Tagged organic materials were retained by interlattice adsorption by all three clays used, although there were no significant differences in retention between clays. Radioactivity was found in clay residues after HF treatment to remove interlattice fractions, and it was found also in the smallest sized particles of clay residues obtained by centrifu-

gation after removal of interlattice fractions. Amino acids containing S-35 were present in interlattice fractions removed from montmorillonite clay.

Inorganic S-35 and C-14 were retained by clay residues after removal of three fractions and three clay particle size separations. There was a greater retention of inorganic S-35 and C-14 than organically combined S-35 and C-14 by clay residues after removal of water-soluble and surface-adsorbed fractions. The retention of organically combined S-35 and C-14 by clay residues, however, was greater after removal of interlattice fractions and in the smallest size particles.

The retention of tagged organic matter and inorganic S-35 and C-14 by sterilized clays did not differ greatly from unsterilized clays, although there was generally less retention of inorganic S-35 and C-14 by the sterilized clays. This may be an indication that microorganisms increased retention of the inorganically added radioisotopes in unsterilized clays.

Further investigations should be conducted on effects of heating clays on lattice collapse and fixation, kinds of organic materials fixed within clay interlayers, and fixation of metabolic products of microorganisms.

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APPENDIX

Table 1—Data for constructing calibration curve for self-absorption of radioactivity by samples of montmorillonite clay treated with organically combined S-35 after removal of water-soluble fraction.

Mass of samples	Observed activity	Apparent specific activity	Per cent of normal
mg.	cpm	cpm/100 mg.	%
2.2	30	1364	100
17.1	215	1257	92
38.6	371	961	70
61.5	542	881	65
91.9	498	541	40
136.2	535	393	29
178.5	605	339	25
185.0	527	285	21
203.1	550	271	20

Table 2—Retention of Rb-86 by clays obtained from Merrimac and Suffield soils and differentially heated, trial one, February 20, 1963.

Clay	Temperature	Radioactivity in filtrate		Retention of Rb-86
		Gross	Net	
	C.	cpm	cpm	%
Merrimac	20	85	26	86.4
"	200	69	10	94.8
"	400	66	7	96.3
'	600	110	51	73.3
'	800	130	71	50.6
Suffield	20	74	15	92.1
"	200	76	17	90.5
"	400	59	0	100.0
	600	84	25	85.6
"	800	178	119	21.1
Standard	20	250	191	—

Table 3—Retention of Rb-86 by clays obtained from Merrimac and Suffield soils and differentially heated, trial two, March 1, 1963.

Clay	Temperature	Radioactivity in filtrate		Retention of Rb-86
		Gross	Net	
	C.	cpm	cpm	%
Merrimac	20	81	12	86.7
	200	73	4	95.6
"	400	73	4	95.6
	600	105	36	60.0
	800	116	47	47.8
Suffield	20	77	8	91.1
	200	72	3	96.7
	400	70	1	98.9
	600	78	9	90.0
	800	132	63	30.0
Standard	20	159	90	—

Table 4—Summary table for analysis of variance of data[1] obtained from two trials on retention of Rb-86 by clays obtained from Merrimac and Suffield soils and differentially heated.

Source	D.F.	M.S.
Total	19	
Trials	1	0.0259
Clays (C)	1	29.9635
Temperature (T)	4	1245.3437**
C x T	4	122.6293**
Experimental Error	9	11.8446

[1] Analysis conducted using arcsin transformation of data.

** p .01

Table 5—Retention of organically combined S-35 by residues of three clays after each removal of six fractions, average of three runs and duplicate samples, 1964.

Residues	Clays		
	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	155,503	143,275	195,800
KOH	74,834	71,561	87,818
HF	39,534	39,726	39,144
HF_1	15,522	15,871	9,670
HF_2	11,056	10,952	8,124
HF_3	4,988	3,124	2,715

Table 6—Retention of inorganic S-35 by residues of three clays after each removal of six fractions, average of three runs and duplicate samples, 1964.

Residues	Clays		
	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	309,646	237,659	262,285
KOH	141,941	184,021	185,467
HF	3,723	3,048	5,032
HF_1	2,506	2,900	2,257
HF_2	2,462	2,273	3,144
HF_3	1,787	1,916	2,438

Table 7—Summary table for analysis of variance for data obtained from three runs on retention of inorganic S-35 and organically combined S-35 by three clays.

Source of variation	D.F.	M.S.
Total	215	
Runs	2	0.5732
Residues (R)	5	22.2230**
Clays (C)	2	0.0268
Treatments (T)	1	8.6563**
R x C	10	0.0861
R x T	5	2.5211**
C x T	2	0.1165
R x C x T	10	0.1227
Experimental Error	70	0.2239
Sampling Error	108	0.0402

** p = .01

Table 8—Retention of organically combined S-35 by residues of three sterilized clays after each removal of six fractions, average of duplicate samples, 1964.

	Clays		
Residues	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	115,348	115,444	121,950
KOH	82,132	74,890	103,348
HF	14,710	13,168	18,542
HF_1	27,928	1,590	8,554
HF_2	13,373	2,000	11,084
HF_3	2,072	8,048	5,856

Table 9—Retention of inorganic S-35 by residues of three sterilized clays after each removal of six fractions, average of duplicate samples, 1964.

	Clays		
Residues	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	280,791	289,032	247,357
KOH	126,372	116,010	166,816
HF	4,795	1,686	1,350
HF_1	1,072	—	3,614
HF_2	3,241	1,120	2,626
HF_3	608	928	1,512

Table 10—Summary table for analysis of variance for data obtained on retention of inorganic S-35 and organically combined S-35 by three sterilized clays.

Source of variation	D.F.	M.S.
Total	71	
Residues (R)	5	18.2722**
Clays (C)	2	1.6744
Treatments (T)	1	7.3356*
R x C	10	3.7797**
R x T	5	2.7413*
C x T	2	0.1472
Experimental Error	10	0.7695
Sampling Error	36	1.9691

* $p = .05$
** $p = .01$

Table 11—Retention of organically combined C-14 by residues of three clays after each removal of six fractions, average of three runs and duplicate samples, 1964.

Residues	Clays		
	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	9,145	6,406	8,230
KOH	4,577	4,372	7,867
HF	4,508	5,880	4,359
HF_1	3,825	3,923	2,898
HF_2	2,504	3,884	1,974
HF_3	1,684	1,860	1,609

Table 12—Retention of inorganic C-14 by residues of three clays after each removal of six fractions, average of three runs and duplicate samples, 1964.

Residues	Clays		
	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	63,275	231,004	140,088
KOH	63,442	223,452	127,580
HF	2,273	2,474	1,201
HF_1	1,064	795	1,230
HF_2	2,504	3,884	568
HF_3	521	1,346	701

Table 13—Summary table for analysis of variance for data obtained from three runs on retention of inorganic C-14 and organically combined C-14 by three clays.

Source of variation	D.F.	M.S.
Total	215	
Runs	2	1.9950*
Residues (R)	5	15.1076**
Clays (C)	2	0.5222
Treatments (T)	1	0.0691
R x C	10	0.1997
R x T	5	7.8399**
C x T	2	0.2236
R x C x T	10	0.3134
Experimental Error	70	0.4108
Sampling Error	108	0.1663

* p = .05
** p = .01

Table 14—Retention of organically combined C-14 by residues of three sterilized clays after each removal of six fractions, average of duplicate samples, 1965.

	Clays		
Residues	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	13,012	9,526	15,320
KOH	5,371	5,013	6,410
HF	2,590	1,923	1,333
HF_1	1,012	1,500	1,641
HF_2	1,166	846	744
HF_3	474	359	616

Table 15—Retention of inorganic C-14 by residues of three sterilized clays after each removal of six fractions, average of duplicate samples, 1965.

Residues	Clays		
	Montmorillonite	From Merrimac soil	From Suffield soil
	cpm/100 mg.	cpm/100 mg.	cpm/100 mg.
H_2O	72,422	102,089	158,487
KOH	7,436	5,012	7,192
HF	1,308	308	1,012
HF_1	705	269	564
HF_2	90	500	436
HF_3	320	359	756

Table 16—Summary table for analysis of variance for data obtained on retention of inorganic C-14 and organically combined C-14 by three sterilized clays.

Source of variation	D.F.	M.S.
Total	71	
Residues (R)	5	11.2622**
Clays (C)	2	0.0906
Treatments (T)	1	1.4816
R x C	10	0.4481
R x T	5	1.0155
C x T	2	0.6249
Experimental Error	10	0.6154
Sampling Error	36	0.9697

** p = .01